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AGGLUTINATION IN THE GROUP OF FLUORESCENT BACTERIA.*

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VERY little has been done as yet in testing agglutination affinities among large groups of bacteria, although to some writers it has seemed probable that this method would throw light upon the true biological relationships of closely allied organisms. A few groups, however, have been closely examined with a view to the differential value of reciprocal agglutination.

A considerable number of cultures of B. coli isolated from the human intestinal tract were studied by Rothberger[1] and Radzievsky.[2] They draw from their work the conclusion that, as regards agglutination, a uniformity among the different cultures of B. coli tested does not exist.

Durham[3] carried out an extended series of tests in an attempt to differentiate various organisms of the coli-typhoid group. He found that certain cultures of the B. enteritidis type which were to all appearance culturally alike showed no real similarity in their agglutinative reactions. It was also true that members of the B. coli group showed variability in their reactions even to very strong serum. On the other hand, cultures of "B. coli communis verus" and of "B. coli communior" gave positive agglutination tests with the same serum, although they were culturally different. He concludes, therefore, that "it is clear that the clumping reaction is of little value for differentiating and classifying these bacilli in a satisfactory manner" (p. 366).

Theobald Smith,[4] in a valuable series of experiments, approaches the subject of bacterial agglutination as one phase of the relation between host and parasite. Cultures of the hog-cholera group isolated from man, the pig, the guinea pig, and the spermophile were tested as to "agglutination relationships" one to another and to related organisms such as B. typhosus, B. coli, and B.

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icteroides. The results of the tests led him to draw the conclusion that "bacteria isolated from well-defined types of disease, especially those known to invade the blood regularly, are clumped in nearly the same dilution." B. icteroides and one of the hog-cholera cultures, however, though differing culturally and in virulence, are clumped in the same dilution by one another's serum. Agglutination tests were made upon eight different cultures of B. coli, seven of which were isolated from the pig and one from the fowl; five of these were used in immunizing rabbits so as to determine the relation between the cultures. Of these eight cultures, four failed to ferment saccharose, and were hence considered by the author to have approached toward a "more pronounced parasitism." The other four he considered to belong to the true colon group. As a result of the tests, he found that, with one exception, the agglutination relationship runs nearly parallel with the biological characters and the specific parasitism. Finally, he concludes that "close agglutinative affinities may be predicted from close biological and pathogenic relationships."

Not many non-pathogenic bacteria have been tested with reference to their agglutinative reaction, but Kneass and Sailer[5] have recently conducted some experiments with B. subtilis. In this work rabbits were treated by the subcutaneous injection of large numbers of the living bacteria. A high degree of agglutinative power was obtained, serum agglutinating in a dilution of 1:5,000 being secured in eighteen days. In testing the agglutination, the microscopic method with filtered broth cultures was employed. Complete agglutination of B. subtilis was obtained in two hours with a serum dilution of 1:2,500, and slight agglutination with a dilution of 1:5,000, but no reaction with a dilution of 1:10,000. The tests were made simply with the specific organism used in immunizing the rabbit.

AGGLUTINATION OF THE FLUORESCENT BACTERIA.

The writer has had the opportunity of studying the agglutinative reactions of forty-six different cultures belonging to the group of fluorescent bacteria, eighteen of these being non-liquefying and twenty-eight liquefying forms. These organisms had all been isolated from river water. Dr. Jordan,[6] in his paper on "The Kinds of Bacteria Found in River Water," has described the methods employed in isolating and studying the cultures I have employed. All of these fluorescent bacteria, with a very few exceptions, were isolated from forty-eight-hour-old gelatin plates, then "rejuvenated" in broth, 0.5 acid, for three days at 20° C., and finally plated in gelatin, from which agar cultures were made for the further examination of the organisms. Three of the organisms (1040 *y*, 1082 *w*, 1082 *x*) were isolated from the gelatin plate after passing through the dextrose broth fermentation-tube, and two others (1076 *l*, 1080 *m*) were isolated from litmus lactose agar plates after passing through carbol broth. These five forms, however, were "rejuvenated" and treated in the same manner as the other organisms of the group.

An examination of Table III accompanying Dr. Jordan's paper will show the main features of the morphology and biology of the fluorescent group. The liquefying type was easily distinguished from the non-liquefying by growth both in gelatin and milk, since all the forms that liquefied gelatin curdled milk with the production of acid, and then peptonized the casein, while the non-liquefying forms produced a strongly alkaline reaction in milk. Aside from these characteristic changes produced in milk and gelatin, the two groups were very similar, and the individual members of each group were practically identical morphologically and biologically.

These bacteria were all isolated and studied in the fall of 1901, while the present study of their agglutination was begun in the spring of 1903. The cultures originally isolated had been kept in stock in the laboratory under uniform conditions. When first examined by the writer, it was found that the power of production of fluorescent pigment had been lost by twenty-five cultures out of the forty-six. The cultural characteristics in gelatin and milk were studied carefully by the writer in the course of the work on agglutination, and found to agree in the main with those exhibited when first studied in 1901, except that curdling of the milk by the liquefying group did not occur; there was in 1903 simply a slight acidification, and this was at once followed by rapid peptonization of the casein. Even a second rejuvenation of the liquefying cultures failed to cause initial curdling of the milk, such as occurred when the organisms were first isolated and rejuvenated eighteen months previously. There still remained, however, the same sharp line of demarkation between the liquefying and non-liquefying groups in respect to behavior in milk and gelatin; the liquefying forms appeared simply to have lost some of their power to produce acid.

METHODS.

The serum used in the agglutination tests was obtained from rabbits injected with specific organisms. Four rabbits were inoculated; two with culture 238 L_1, one with culture 1119 A, and one with culture 1080 M.

The inoculations were carried out in the following way: A suspension in physiological salt solution of a living forty-eight-hour-old slant agar growth of the organism was injected subcutaneously. Beginning with one-half of the scrapings of a forty-eight-hour-old slant agar growth, the amount injected was increased to the whole agar growth, then to two agar growths, and finally to four agar growths at a single injection. Rabbit No. 1, treated with culture 238 L_1, received twenty injections in the course of two months. Rabbit No. 2, also treated with 238 L_1, received eight injections in one month; in this case growth on two agar tubes was used in the first injection, and on the fifth inoculation four agar growths were used and continued. Rabbits Nos. 3 and 4, inoculated respectively with cultures 1119 A and 1080 M, were treated in the same manner as No. 1, but the inoculations were continued for three months in order to obtain agglutination in dilutions above 1:1,000.

The macroscopic method of testing agglutination was used. The cultures to be tested were grown in neutral sugar-free broth for from twenty-four to forty-eight hours at room temperature. The serum used was diluted with physiological salt solution. The serum was added to a measured quantity of the broth culture, so as to obtain the desired dilution of the serum in contact with the bodies of the bacilli, care being taken to obtain a uniform suspension, so far as this could be determined by the eye. A control-tube was always made of each organism tested, and kept under the same conditions as the other tubes. All these tubes were then incubated for twenty-four hours, but were examined at intervals to note the extent of the clumping. Examinations were made at the end of one-half hour, one hour, two hours, three hours, four hours, and twenty-four hours.

Considerable difficulty was experienced at the outset in obtaining cultures of a uniform turbidity, because this group of bacteria tends to form surface films and a flocculent growth. The films, however, could generally be removed, and after some experience it was found practicable to use cultures at an age when they showed the least amount of flocculent growth. For the same reason four hours was found to be the usual limit at which the degree of agglutination could be best established, as the flocculent growth sometimes present after twenty-four hours obscured the reaction. The stronger sera and the lower dilutions of sera, however, sometimes gave a sharp reaction after twenty-four hours.

The serum lost very little of its agglutinating power after standing two weeks; no older serum was tried. Every time a serum was used after standing, and whenever fresh serum was used, tests were made with the homologous organism as well as with the other organisms, to determine whether the serum had lost any of its agglutinating power. Most of the serum used was not more than two or three days old.

The serum from rabbit No. 1, treated with B fluorescens nonliquefaciens 238 L_1, was first used in testing the agglutination of the other forty-five cultures of the fluorescent group. This culture was primarily selected because it was a thoroughly typical and vigorous form biologically, culturally, and in the production of fluorescence. Tables I and II show that of the eighteen other non-liquefying forms, ten gave positive agglutination; of the twenty-eight liquefying forms, fourteen gave a positive reaction, much variation, however, being shown in the degree of dilution of the serum by which they were clumped. Clumping occurred with 238 L_1 in dilution of its serum 1:10,000, while the limit of agglutination of most of the others was with serum diluted only

1:100. Five gave a positive agglutination with dilution 1:5,000, and five with dilution 1:1,000. The agglutination affinities between the liquefying and non-liquefying groups, and between the members within the groups, do not correspond with the biological characteristics.

It might perhaps be expected, first, that, since the organism used for inoculation belonged to the non-liquefying group, the other members of the non-liquefying group, being to all appearances biologically similar, would show practically the same agglutinative reaction; and, second, that there would be some distinction between the liquefying and non-liquefying groups; but in neither case can any such relation be traced.

In view of the results with immune serum 238 L_1, it was decided to inoculate three other rabbits; rabbit No. 2 with 238 L_1, since rabbit No. 1 had died in the interval; rabbit No. 3 with culture 1119 A, a member of the liquefying group of fluorescent bacteria and one agglutinated by immune serum 238 L_1 in dilution 1:5,000; and rabbit No. 4 with culture 1080 M, a member of the non-liquefying group, but not agglutinated by serum 238 L_1. It was found much more difficult to obtain a powerful serum with cultures 1119 A and 1080 M than it was with 238 L_1, it being necessary to extend the inoculations over a longer period, although a larger number of bacteria were inoculated each time. The writer was unable to raise the strength of the serum of rabbit No. 3, immunized against 1119 A, beyond the point of agglutination with serum diluted 1:4,000, and rabbit No. 4, immunized against 1080 M, beyond serum dilution 1:2,000.

Tests were made with the sera 1119 A and 1080 M on the forty-six fluorescent cultures just as in the case of serum 238 L_1. With serum 1119 A, eight of the eighteen non-liquefying forms gave a positive agglutination reaction, while this was true of only six of the twenty-eight liquefying forms, although 1119 A is a member of the liquefying group. With serum 1080 M, nine of the eighteen non-liquefying forms, and four of the twenty-eight liquefying forms, gave a positive reaction, 1080 M being a member of the non-liquefying group. As in the case of serum 238 L_1, the tests with sera 1119 A and 1080 M show no agglutinative relationships between the liquefying and non-liquefying groups, or between the members within these groups.

Table III shows in comparison the agglutinations produced by the three sera. Serum 238 L_1 agglutinates a larger number and in higher dilution than either of the other two sera, probably because it is the most potent. Serum 1080 M, although somewhat weaker than 1119 A, agglutinates almost exactly the same number of cultures as serum 1119 A, and in about the same dilution. Mutual clumping reactions do not exist between these three cultures; for culture 238 L_1 is not agglutinated by either serum 1119 A or 1080 M; culture 1119 A is not agglutinated by serum 1080 M, but is by 238 L_1; culture 1080 M is not agglutinated by serum 238 L_1, but is by 1119 A. Culture 1119 A is agglutinated by serum 238 L_1 in dilution 1:5,000, while with its own less highly immunized serum it is agglutinated in dilution 1:4,000. The table shows further that not all the cultures agglutinated by serum 238 L_1 were clumped by serum 1119 A or 1080 M, nor were all the cultures agglutinated by serum 1119 A or 1080 M clumped in turn by serum 238 L_1. Nevertheless, it is true that, with a single exception, all the cultures agglutinated by serum 238 L_1, in dilution of 1:1,000, were also clumped by serum 1119 A or 1080 M in high dilution. It would appear that certain of the cultures were more sensitive to agglutination than others. Possibly if a higher immunity had been reached, more cultures would have been agglutinated. Although in general the strongest serum 238 L_1 clumped the cultures in higher dilutions than either serum 1119 A or 1080 M, there were a few exceptions. Culture 243 F_2 was agglutinated with serum 1119 A diluted 1:1,000 and not at all by serum 238 L_1; culture 1091 C was agglutinated by serum 1119 A diluted 1:500, while the stronger serum 238 L_1 agglutinated this culture only in dilution 1:100; culture 1021 D was agglutinated by serum 1080 M diluted 1:1,000, and by serum 238 L_1 diluted 1:100.

Table IV gives the varying degrees of agglutination shown by the three specific organisms used in these experiments. Complete agglutination, or that in which the clumped bacteria are completely precipitated, occurs at a lower dilution than the point of just visible agglutination, or that in which the slightly clumped bacteria remain still suspended in the fluid.

To determine whether normal rabbit serum contained agglutinins sufficient to clump the fluorescent group of bacteria, tests were made with the serum of three different rabbits upon cultures 1119 A and 238 L_1 with negative results in every case, although low dilutions, 1:10 and 1:50, of the serum were used.

At the time of these agglutination experiments, there were in stock in the laboratory seven Pyocyaneus cultures from various sources, and five cultures of the fluorescent group which had been isolated from the human stomach. Agglutination tests were made with these organisms, using the three immune sera as in the other experiments, and showed that only one Pyocyaneus culture was clumped, and that only slightly, by serum 238 L_1 in dilution 1:100. In view of the small number of cultures tested from the Pyocyaneus group, no definite conclusion is justified. Of the five fluorescent cultures isolated from the human stomach, two of the three non-liquefying forms were slightly clumped by the serum of 238 L_1 (1:100).

I wish to express my thanks to Dr. Jordan, under whose direction this work was undertaken and carried on, for his valuable suggestions, and especially for his guidance and encouragement toward independent work.

CONCLUSIONS.

1. In the case of the group of fluorescent bacteria there appears to be no definite relation between the biological characteristics and the agglutinative reaction.

2. Certain of the cultures are more sensitive to agglutination than others, for, with one exception, the cultures agglutinated by serum 238 L_1 diluted 1:1,000 + were agglutinated by sera 1119 A and 1080 M in high dilution.

3. The strongest serum agglutinates the largest number of cultures and in the highest dilution.

4. These non-pathogenic organisms are less sensitive to the agglutination test than the parasitic and semi-parasitic bacteria of the colon-typhoid group.

TABLE I.

Agglutination of the Non-Liquefying Group of Fluorescent Bacteria by the Serum of a Rabbit Immunized to B. fluorescens non-liquefaciens 238 L$_1$.

NAME	DILUTION 1:40					DILUTION 1:100					DILUTION 1:1,000					DILUTION 1:5,000					DILUTION 1:10,000				
	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.
238 L$_1$	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+ +	+ +	+ +	+	+	+	+	+	–	–	+ –	+ –	+
1070 D	+ –	+ –	+ –	+	+	+ –	+ –	+ –	+ –	+	–	–	–	–	+ –	–	–	–	–	+ –	–	–	–	–	–
198 L	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–
2121 A$_1$	–	–	+ –	+	+	–	–	+ –	+	+	–	–	–	+ –	+ –	–	–	–	–	–	–	–	–	–	–
2121 A$_2$	–	–	–	–	+ –	–	–	–	–	+ –	–	–	–	–	+ –	–	–	–	–	–	–	–	–	–	–
225 B$_2$	–	–	+ –	+ –	+ –	–	–	+ –	+ –	+ –	–	–	–	+ –	+ –	–	–	–	–	–	–	–	–	–	–
225 B$_1$	–	–	+	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
173 B	–	–	+	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
116 G	–	–	+ –	+ –	+	–	–	+ –	+ –	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1082 W	–	–	–	–	+ –	–	–	–	–	+ –	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
116 E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2113 F$_1$	–	–	–	+ –	+ –	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2113 F$_2$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2113 F$_3$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
114 A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
180 X	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2102 F$_2$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
243 F$_2$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE II.

Agglutination of the Liquefying Group of Fluorescent Bacteria by the Serum of a Rabbit Immunized to B. fluorescens non-liquefaciens 238 L$_1$.

NAME	DILUTION 1:40					DILUTION 1:100					DILUTION 1:1,000					DILUTION 1:5,000					DILUTION 1:10,000				
	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.
288 L$_2$	–	+ +	+ +	+ +	+ +	–	+ +	+ +	+ +	+ +	–	+	+ +	+ +	+ +	–	–	+	+	+	–	–	–	–	–
1119 A	–	+ –	+ –	+ –	+	–	+ –	+ –	+ –	+	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–
185 Z	–	+ –	+ –	+ +	+ +	–	+ –	+ –	+	+	–	–	–	+	+	–	–	–	+ –	+ –	–	–	–	–	–
1022 B	–	–	–	+	+	–	–	–	+	+	–	–	–	+	+	–	–	–	+ –	+ –	–	–	–	–	–
2081 D$_2$	–	–	–	–	+	–	–	–	–	+	–	–	–	–	+ –	–	–	–	–	–	–	–	–	–	–
1021 D fl	–	–	–	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1092 A	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1040 Y	–	–	–	+	+	–	+ –	+ –	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2049 A	–	–	+	+	+ –	–	–	+ –	+ –	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE II—*Continued.*

NAME	DILUTION 1:40					DILUTION 1:100					DILUTION 1:1,000					DILUTION 1:5,000					DILUTION 1:10,000				
	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.
1091 C..	—	—	—	—	±	—	—	—	—	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1043 D..	±	±	±	±	±	±	±	±	±	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1069 A..	—	—	±	±	±	—	—	±	±	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2099 A..	—	—	—	±	±	—	—	—	—	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1070 A..	—	+	+	+	+	—	±	±	±	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
82 B..	—	—	—	—	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1092 B..	—	—	—	—	+ 24 hrs	—	—	—	—	+ 24 hrs	—	—	—	—	+ 24 hrs	—	—	—	—	—	—	—	—	—	—
1043 B..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2075 D..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1015 A..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2073 E..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1076 L..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2073 E..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2099 D..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1079 A..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1082 X..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1080 M..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1044 W.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1059 B..	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE III.

Comparison of the Agglutination of the Fluorescent Group of Bacteria Produced by Sera from Three Rabbits Immunized Respectively to 238 L_1, 119 A, and 1080 M.

FLUORESCENS NON-LIQUEFACIENS	LIMIT OF AGGLUTINATION WITH			FLUORESCENS LIQUEFACIENS	LIMIT OF AGGLUTINATION WITH		
	Serum 238 L_1	Serum 1119 A	Serum 1080 M		Serum 238 L_1	Serum 1119 A	Serum 1080 M
238 L_1	10,000	(No aggl.)	(No aggl.)	238 L_2	5,000	(No aggl.)	(No aggl.)
1070 D	5,000±	(No aggl.)	1,000	1119 A	5,000	4,000	(No aggl.)
2121 A_1	1,000±	1,000	1,000	185 Z	5,000±	1,000	1,000
2121 A_2	1,000±	1,000	1,000	1022 B	5,000±	1,000	1,000
198 L	1,000	1,000	(No aggl.)	2081 D_2	1,000±	(No aggl.)	(No aggl.)
225 B_2	1,000±	1,000±	1,000	1021 D fl	100	(No aggl.)	1,000
225 B_1	100	(No aggl.)	(No aggl.)	1092 A	100	(No aggl.)	(No aggl.)
173 B	100	(No aggl.)	(No aggl.)	1040 Y	100	100±	(No aggl.)
116 G	100	(No aggl.)	(No aggl.)	2049 A	100±	(No aggl.)	(No aggl.)
1082 W	100±	100±	100±	1091 C	100±	500	(No aggl.)
243 F_2	(No aggl.)	1,000	100	1043 D	100±	(No aggl.)	100±
1080 M	(No aggl.)	500±	2,000	1069 A	100±	100±	(No aggl.)
114 A	(No aggl.)	40	40	2099 A	100±	(No aggl.)	(No aggl.)
1082 X	(No aggl.)	(No aggl.)	100	1070 A	100±	(No aggl.)	(No aggl.)

TABLE IV.

Degrees of Agglutination of 238 L_1, 1119 A, and 1080 M by Their Respective Sera.

Dilution	hrs.	238 L_1		1119 A		1080 M	
1:40	½	+++	Complete	—		—	
	1	+++	Complete	++	Strong	+	Visible
	2	+++	Complete	+++	Complete	+++	Complete
	3	+++	Complete	+++	Complete	+++	Complete
	4	+++	Complete	+++	Complete	+++	Complete
	24	+++	Complete	+++	Complete	+++	Complete
1:100	½	+	Visible	—		—	
	1	+	Visible	+	Visible	+	Visible
	2	+++	Complete	+++	Complete	+++	Complete
	3	+++	Complete	+++	Complete	+++	Complete
	4	+++	Complete	+++	Complete	+++	Complete
	24	+++	Complete	+++	Complete	+++	Complete
1:200	½	+—	Slight	—		—	
	1	+	Visible	+	Visible	+	Visible
	2	+++	Complete	+++	Complete	+++	Complete
	3	+++	Complete	+++	Complete	+++	Complete
	4	+++	Complete	+++	Complete	+++	Complete
	24	+++	Complete	+++	Complete	+++	Complete
1:400	½	—		—		—	
	1	+	Visible	—		—	
	2	+	Visible	—		—	
	3	+++	Complete	+	Visible	+—	Slight
	4	+++	Complete	+	Visible	+	Visible
	24	+++	Complete	+	Visible	++	Strong
1:600	½	—		—		—	
	1	—		—		—	
	2	+	Visible	—		—	
	3	++	Strong	—		+—	Slight
	4	++	Strong	+	Visible	+	Visible
	24	+++	Complete	+	Visible	++	Strong
1:800	½	—		—		—	
	1	—		—		—	
	2	+—	Slight	—		—	
	3	+	Visible	—		+—	Slight
	4	+	Visible	+	Visible	+	Visible
	24	++	Strong	+	Visible	++	Strong
1:1,000	½	—		—		—	
	1	—		—		—	
	2	+	Visible	—		—	
	3	+	Visible	+	Visible	+	Visible
	4	+	Visible	++	Strong	+	Visible
	24	++	Strong	++	Strong	+	Visible
1:2,000	½	—		—		—	
	1	—		—		—	
	2	—		—		—	
	3	—		—		+—	Slight
	4	+	Visible	+	Visible	+	Visible
	24	+	Visible	+	Visible	+	Visible

TABLE IV.—*Continued.*

Dilution	hrs.	238 L$_1$		1119 A		1080 M	
1:4,000	½	—		—		—	
	1	—		—		—	
	2	—		—		—	
	3	+	Visible	—		—	
	4	+	Visible	+	Visible	—	
	24	+	Visible	+	Visible	—	
1:5,000	½	—		—		—	
	1	—		—		—	
	2	—		—		—	
	3	—		—		—	
	4	+—	Slight	—		—	
	24	+	Visible	—		—	

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